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PATENT & TRADEMARK OFFICE
1637

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Shigeru KANAOKA
Serial No.: 10/549,389
Filed: September 14, 2005
For: **METHOD OF DETECTING COLON CANCER MARKER**
Art Unit: 1637
Examiner: PANDE, SUCHIRA

Honorable Commissioner of Patents
and Trademarks
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 CFR 1.132

SIR:

I. I, Shigeru KANAOKA, an inventor of this case, declare and say as follows.

I am the inventor of the present U.S. Patent Application as identified above and understand the English language. I graduated Hamamatsu University School of Medicine in March, 1987, with the degree of M.D. Since then, I have been working on clinical research of gastroenterology and basic research of gastrointestinal oncology. In the meantime, I had been an assistant professor of First Dept. of Medicine since 2000, and I have been a professor of Dept. of Molecular Diagnosis since 2007 at Hamamatsu University School of Medicine. Early 1997 I was given a Ph.D. in gastroenterology for my work on colon carcinogenesis. I studied the Official Action dated November 26, 2008 received in the present application.

In order to show that the present invention is not obvious over the references cited by the Examiner, I have

conducted comparative experiments as mentioned below under my supervision.

II. Comparative data

In Final rejection, based on the small data set provided it appears to the Examiner that the data did not show any unexpected improvement over detection of colon cancer using CEA and occult blood. I would like to draw the Examiner's attention to the unexpected result of the applicant's amended claims over the references cited by the Examiner. Therefore I increased the number of cases and controls to provide comparative data regarding sensitivity and specificity among the five colon cancer detecting methods (COX-2, CEA, occult blood, E1AF, c-myc).

EXPERIMENT

(1) Materials and Methods

Among patients hospitalized in the First Department of Internal Medicine of Hamamatsu University School of Medicine for detailed examination and therapy, 70 patients with colorectal cancer (CRC) who were diagnosed colonoscopically and histologically were selected as the studied population, and a total of 34 subjects in whom no pathological findings were observed were served as controls. Informed consents of all the cases had been obtained.

Stool samples were collected at between two weeks and four weeks after diagnostic colonoscopy with a few biopsies. Stool sample collection was done before endoscopic or surgical resection of CRC. Collected samples were stored at 4°C immediately after collection and transferred to a -80°C freezer within 24 hours. Also, for comparison and reference, human hemogolobin (Hb) in the feces of each sample was measured by the immunological fecal occult blood test. Then, feces were homogenized using a homogenizer, guanidine salt,

and phenol, and whole RNA was extracted using chloroform and ethanol. One micro gram of the obtained RNA was reverse transcribed using ReverScript II (a registered trade mark), (reaction mixture volume: 20 micro litter, Wako Pure Chemical Industries) to give cDNA. A part thereof was amplified by means of nested PCR using GeneTaq (Wako).

(2) Results

(a) Comparison of COX-2 assay between CEA assay and fecal occult blood test

The results were shown in the following table.

marker	sensitivity	specificity
COX-2	85.7% (60/70)	100.0% (34/34)
CEA	92.9% (65/70)	11.8% (4/34)
occult blood	73.5% (50/68)	82.4% (28/34)

The CEA assay had higher sensitivity than the occult blood test (P=0.0048, by the chi-square test), however, it had lower specificity than the occult blood test (P<0.0001, by the chi-square test). These results meant that the studied population consisted of 70 patients with CRC and 34 control subjects were enough to judge the effectiveness of the methods.

Although the COX-2 assay did not have higher sensitivity than the CEA assay, the difference was not significant (P=0.27, by the chi-square test). The COX-2 assay had significantly higher specificity than the CEA assay (P<0.0001, by the chi-square test). The COX-2 assay had higher sensitivity than the occult blood test, but the difference was not significant (P=0.12, by the chi-square test). However, COX-2 assay had significantly higher specificity than the occult blood test (P=0.033, by the chi-square test; ref 1).

The Examiner deemed that CEA is not shown to be a valid marker for colon cancer detection. However, it is reported that fecal CEA is useful for detecting CRC (ref 2, 3).

(b) Comparison among COX-2 E1AF and c-myc assays

Next I analyzed other markers other than COX-2 and CEA. I selected two markers, Ets-related transcriptional factor (E1AF) and c-myc (ref 4, 5) that are reported to involve in colon carcinogenesis. The results were shown in the following table.

marker	sensitivity	specificity
COX-2	85.7% (60/70)	100.0% (34/34)
E1AF	27.5% (19/69)	96.7% (29/30)
c-myc	42.9% (15/35)	83.3% (20/24)

The COX-2 assay had higher specificity than the both E1AF and c-myc assays, but the difference was not significant ($P=0.95$, $P=0.052$, by the chi-square test, respectively). However, The COX-2 assay had significantly higher sensitivity than the both assays ($P<0.0001$, $P<0.0001$, by the chi-square test, respectively).

Reference

1) Shigeru Kanaoka, Tetsunari Takai, Ken-ichi Yoshida, Yasushi Hamaya, Mutsuhiro Ikuma, Akira Hishida: A Comparison of Fecal RNA Test with Immunochemical Fecal Occult Blood Test for Detecting Colorectal Cancer and Adenoma. Gastroenterology 2007;132(4)Suppl. 1: A-623.

2) Kim Y, Lee S, Park S, et al. Gastrointesitinal tract cancer screening using fecal carcinoembryonic antigen. Ann clin Lab Sci 2003; 33:32-38.

3) Stubbs RS, Nadkarni DM, Monsey HA. Fecal carcinoembryonic antigen in colorectal cancer patients. Gut 1986;26:901-905.

4) Nosho K, Yoshida M, Yamamoto H, et al. Association of Ets-related transcriptional factor E1AF expression with

overexpression of matrix metalloproteinases, COX-2 and iNOS in the early stage of colorectal carcinogenesis. Carcinogenesis 2005;26:892-899.

5) Lagerholm S, Dutta S, Nair P. Non-invasive detection of c-myc p64, c-myc p67 and c-erbb-2 in colorectal cancer. Scand J Gastroenterol 2005;40:1343-50.

(3) Consideration

Since it is very important for the screening test to have both high sensitivity and high specificity. The results of (a) especially showed unexpected improvement over the conventional fecal occult blood test for detection of colon cancer in terms of specificity. Similarly, The results of (b) showed that the COX-2 assay had high sensitivity for detecting colorectal cancer with maintaining high specificity compared with other markers.

III. Conclusion

I believe that the above results would indeed be surprising and could never be expected from the description of the cited reference. Thus, I do not believe that the present invention is obvious over the reference cited by the Examiner.

IV. I further declare that all statements made herein of may own knowledge are true and that all statements made in information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: April 23, 2009

Shigeru Kanaoka

Shigeru Kanaoka